

West Virginia Office of Laboratory Services

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Microbiology- Tuberculosis Unit



Objectives

At the end of this presentation participants will be able to:

- Identify symptoms of TB
- Describe the Office of Lab Services role in TB Control
- Describe the proper procedure for specimen transport
- Identify acceptable specimens for TB testing
- Identify requirements for MTD (Mycobacterium Tuberculosis Direct) testing to be performed
- Describe the types of testing performed at OLS for TB
- Explain why some testing is referred to outside laboratories

Introduction to Tuberculosis

- What is TB?
 - Mycobacteria are part of the bacteria family
 - Cause a variety of diseases
 - Some may be similar to TB
 - *M. bovis*, *M. africanum*, *M. microtti*, and *M. canetti* can cause tuberculous disease
 - In U.S. the majority of TB cases are caused by the *Mycobacterium tuberculosis* organism

How TB is Spread

- Through the air from person to person
- Person with infectious TB disease coughs or sneezes
- Tiny particles called droplet nuclei containing *M. tuberculosis*, are expelled into the air
- Transmission occurs when another person inhales air containing these droplet nuclei

Four Factors Determine the Probability of TB Being Transmitted

- How infectious the TB patient is
- Environment
- Length of Exposure
- How pathogenic the tubercle bacilli (organism) are

Close Contacts

Close contacts to a TB patient are at the highest risk of contracting TB disease

- Family members
- Roommates
- Friends
- Coworkers
- Classmates
- Etc.

Stopping Transmission

- Isolation of infectious person
- Start standard TB treatment as soon as possible
- Length of time for treatment varies from person to person
- If therapy is followed correctly, a rapid decline in infectiousness of patient should occur

Latent TB Infection (LTBI)

- Tubercle bacilli are in the body but the immune system is keeping them under control
- Detected by
 - TST -Tuberculin Skin Test
 - Mantoux TST
 - IGRA- interferon-gamma release assay
 - QuantiFERON Gold (blood test)
- Person is **NOT** considered infectious

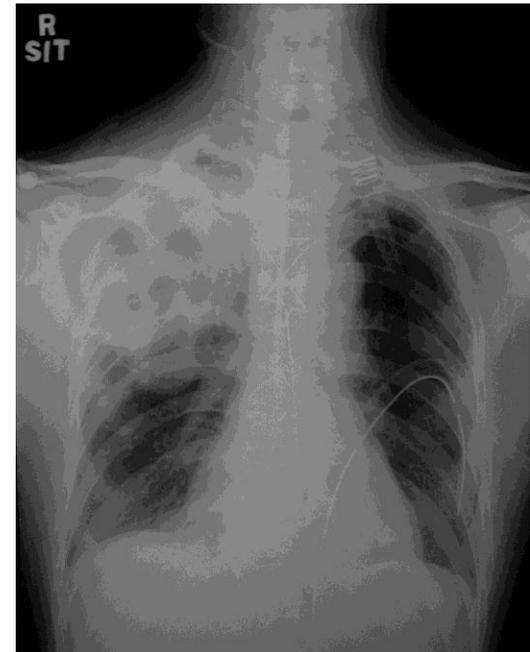


TB Disease

- Approximately 10% of all people with normal immune systems and LTBI develop TB disease
- 90% will remain infected but not develop active disease
- Health risks increase chance of developing active TB
 - HIV infection, Diabetes, kidney disease, certain types of cancer, substance abuse, etc.

Symptoms of TB Disease

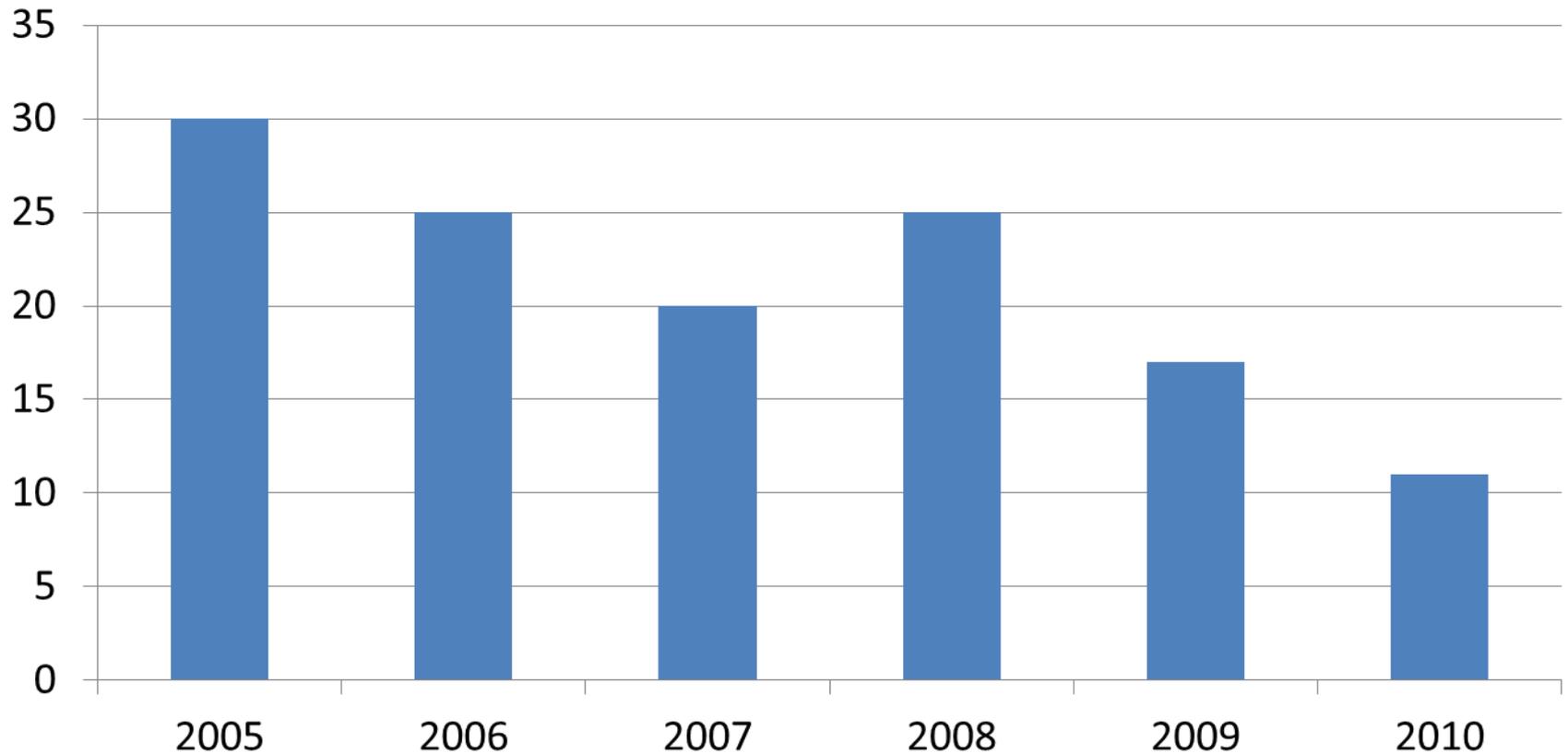
- Cough (lasts more than 3 weeks)
- Chest pain when breathing
- Coughing up sputum or blood
- Fever
- Chills
- Night sweats
- Weight loss
- Fatigue
- Abnormal x-ray of lungs



Worldwide Significance

- Estimated 2 billion people are infected with TB worldwide
- One of the leading causes of death in people older than 5 years of age
- All TB cases are required to be reported to local TB control
 - Treatment
 - Contact Investigation

West Virginia TB Cases



OLS Specimen Numbers

2009

- 1,186 samples tested
- 765 individual patients
- 10 TB culture positive
- 110 Reference isolates
- 13 sent for DST
- 117 MTD tests
- 4 MTD TB positive
- 17 Fingerprint

2010 to date

- 1,146 samples tested
- 762 individual patients
- 9 TB culture positive
- 132 Reference isolates
- 10 sent for DST
- 109 MTD tests
- 3 MTD TB positive
- 11 Fingerprint

OLS Services Provided To

- WV County Health Departments
- Hospitals
- Correctional Facilities
- Reference Labs
 - Any culture that is positive for *M. tuberculosis* at another facility must be sent to OLS for confirmation, via biochemical test, and referral for Fingerprint
 - OLS is the only facility that can submit isolates for FP for the state of WV for Genotyping

Role of the Lab in TB Control

Work in correlation with WV TB Controllers

- OLS reports all new positive smears, MTD, TB complex probes and DST results by phone and hard copy to TB Control and the submitter
- Reported within 24 hours of detection by lab
- Allows for proper treatment, isolation of patient and/or DOT to be administered

TB Control

- Informs the local Health Department
 - Conducts patient interview
 - Begins contact investigation
- Contacts patients private physician and/or Infection Control at the facility where the patient is hospitalized

TB Control Cont.

- Collect reporting info for TB Register and CDC from appropriate agency (hospital, LHD, clinic)
- Follow up with Health Dept. or Physician
 - Started on medications
 - Receiving appropriate treatment
 - Isolation
 - Collecting sputum/ specimens
 - Chest x-rays, etc.

Specimen Collection

- Kits available by request from OLS
- Request forms available on OLS website
<http://www.wvdhhr.org/labservices/>
 - Microbiology Specimen Kit Requisition Form
 - Microbiology Laboratory Specimen Submission Form
- Patient collection instructions available on website under Labs/TB
 - Available in English and Spanish

MICROBIOLOGY SPECIMEN KIT REQUISITION FORM

REQUEST FROM:			
NAME OF FACILITY			
MAILING ADDRESS	CITY	STATE	ZIP
NAME OF PERSON REQUESTING KITS		TITLE	
PHONE NUMBER		DATE	

MAILING KIT	QUANTITY	
	ORDERED	SENT
Stool (Fecal) Culture -- CARY BLAIR <i>USE: For screening stool specimens for the presence of enteric bacteria</i> SUPPLIED IN QUANTITIES OF TEN (10) PER BOX		
Parasitology -- 10% FORMALIN <i>USE: For screening stool for presence of parasites</i> SUPPLIED IN QUANTITIES OF TEN (10) PER BOX		
Pinworm <i>USE: For screening cellulose tape mounts for the presence of pinworms or pinworm eggs</i> EACH		
Mycobacteriology (TB) <i>USE: For screening clinical specimens for the presence of Mycobacterium tuberculosis</i> SUPPLIED IN QUANTITIES OF TEN (10) PER BOX		
Pertussis -- REGAN-LOWE <i>USE: For screening nasopharyngeal swabs for the presence of Bordetella pertussis</i> EACH		
Norovirus *** <i>USE: For screening stool specimens for the presence of Noroviruses</i> *** Collection kits for Norovirus testing REQUIRE prior consultation and approval of Section supervisor. Please contact the number below.		

ADDITIONAL SUPPLIES		
Mycobacteriology (TB)		
Inner bag kits, each kit includes clear plastic bag (1) and white Tyvek bag (1)		

NOTE: Most kits contain specimen collection vial/tube, absorbent sheet, zippered plastic bag, inner and outer mailer, and test request form.

CONTACT INFORMATION	
Microbiology Section	2602
TB Unit	2621
Molecular Unit	2141
Microbiology Supervisor	2610
Container Room	2204

Order Filled By: _____
Order Shipped By: _____
Date: _____

STATE OF WEST VIRGINIA • DEPARTMENT OF HEALTH AND HUMAN RESOURCES • BUREAU FOR PUBLIC HEALTH
 OFFICE OF LABORATORY SERVICES
 167 11th AVENUE
 SOUTH CHARLESTON, WV 25303
 PHONE (304) 338-3330 • FAX (304) 338-6210



PLACE BARCODE HERE

OLS USE ONLY

MICROBIOLOGY LABORATORY SPECIMEN SUBMISSION FORM

PATIENT INFORMATION

PATIENT ID (Chart #, etc.)		
LAST NAME	FIRST NAME	MI
DATE OF BIRTH	SS# (last 4 only, optional)	
COUNTY OF RESIDENCE	SEX <input type="checkbox"/> Female <input type="checkbox"/> Male	
STREET ADDRESS		
CITY	STATE	ZIP
PATIENT PHONE NO. (optional)		

DATE OF COLLECTION:

SITE/SOURCE OF SPECIMEN:

<input type="checkbox"/> Blood	<input type="checkbox"/> Sputum
<input type="checkbox"/> Cellulose tape mount	<input type="checkbox"/> Sputum, induced
<input type="checkbox"/> CSF	<input type="checkbox"/> Stool
<input type="checkbox"/> Nasopharyngeal	<input type="checkbox"/> Stool, bloody
<input type="checkbox"/> Rectal	<input type="checkbox"/> Throat
<input type="checkbox"/> Serum	<input type="checkbox"/> Urethra
<input type="checkbox"/> Serum, acute	<input type="checkbox"/> Urine
<input type="checkbox"/> Serum, convalescent	
<input type="checkbox"/> Wound Location:	
<input type="checkbox"/> Bronchial	Specify:
<input type="checkbox"/> Tissue	Specify:
<input type="checkbox"/> Fluid	Specify:
<input type="checkbox"/> Other	Specify:

SUBMITTER INFORMATION

FACILITY NAME		
MAILING ADDRESS		
CITY	STATE	ZIP
COUNTY		
ATTENTION TO		
PHONE NO.		
FAX NO.		

COMMENTS:

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OLS USE ONLY

<input type="checkbox"/> UNSAT Reason:	ACC:
<input type="checkbox"/> UNRELIABLE Reason:	DE:
<input type="checkbox"/> SATISFACTORY	KCD:

TEST(S) REQUESTED:

BACTERIOLOGY	MYCOBACTERIOLOGY
<input type="checkbox"/> Referred Culture	<input type="checkbox"/> Culture/Smear *
<input type="checkbox"/> Pertussis culture / PCR	<input type="checkbox"/> TB ID/Confirmation *
<input type="checkbox"/> Enteric (stool in Cary-Blair)	<input type="checkbox"/> MOTT Identification *
<input type="checkbox"/> Gonorrhea culture	Suspected Organism:
<input type="checkbox"/> Gonorrhea smear	Date growth appeared:
<input type="checkbox"/> Unknown bacteriology ID	Patient taking TB drugs? <input type="checkbox"/> Yes <input type="checkbox"/> No
Suspected Organism (s):	Date Started:

VIROLOGY

<input type="checkbox"/> Influenza RT-PCR
Submitted for:
<input type="checkbox"/> Surveillance
<input type="checkbox"/> Sentinel Provider / Hosp
<input type="checkbox"/> Other *
(note in Comments)
<input type="checkbox"/> Outbreak
If outbreak ...
<input type="checkbox"/> School
<input type="checkbox"/> Nursing Home
<input type="checkbox"/> Other
County: _____
Was sample frozen? <input type="checkbox"/> Yes <input type="checkbox"/> No

<input type="checkbox"/> Norovirus RT-PCR**
EPI Contact Name:

<input type="checkbox"/> Arbovirus, human (Date of Symptom Onset: _____)
--

Skin Test <input type="checkbox"/> POS (+) <input type="checkbox"/> NEG (-)
Chest X-ray <input type="checkbox"/> Abnormal <input type="checkbox"/> Normal
Contact to TB patient? <input type="checkbox"/> Yes <input type="checkbox"/> No
Refrigerated? <input type="checkbox"/> Yes <input type="checkbox"/> No

PARASITOLOGY
<input type="checkbox"/> Fecal Parasite Exam 10% formalin
<input type="checkbox"/> Fecal Parasite Exam PVA
<input type="checkbox"/> Pinworm Exam

* "Others" MUST be approved by DDC.

** Norovirus testing performed on outbreak specimens ONLY.

Specimen Collection Cont.

Kit consists of:

- Sterile 50ml conical tube
- Inner clear plastic bag
- Absorbent material
- Outer white Tyvek bag
- Shipping container with UN3373 label Cat. B
- Collection instructions
- Business reply label for Health Departments
- Laboratory Specimen Submission Form

Specimen Collection Cont.

Includes filling Specimen Submission Form out correctly

- Specimens ***will be*** rejected for incomplete submission forms in all areas of OLS Lab
- Delays in testing and results

Very important to package specimens properly to prevent exposure to OLS staff.

- Nurses at Health Departments need to instruct patients on home collection and transport

Specimen Transport

- Samples should be mailed on day of collection
- Delays in transport will allow for growth of contaminating bacteria
- If transport is delayed for more than 1 hour, samples should be refrigerated and mailed as soon as possible
 - Form should be marked **Refrigerated** so specimens are not considered unreliable or unsatisfactory due to transit time

Personal Protective Equipment



Acceptable TB Specimens

- Sputum, induced sputum
- Bronchial (wash or lavage)
- Cerebral Spinal Fluid (CSF)
- Fluid (pleural, Synovial joint, etc.)
- Tissue
- Urine

Specimen Condition

- **Satisfactory**
 - Received within 2 days of collection

- **Satisfactory-refrigerated**
 - Refrigerated and date of collection is greater than 2 days

Specimen Condition

- Unreliable

- Less than 5 ml, sputum only
- 3-4 days after collection without refrigeration
- Unknown date of collection
- Resembles saliva
 - Accepted in very young and elderly

Specimen Condition

- Unsatisfactory
 - In transit 5 days
 - Insufficient amount, less than 1 ml
 - Leaked in transit
 - No specimen received
 - No name (unlabeled)

Specimens that are unsatisfactory
will NOT be processed.

Proper Amount of Specimen

- Unreliable Specimens
 - Cannot be counted by TB Control to document conversion to negative
 - Transit time (greater than 2 days without being refig.) and less than 5 ml of sputum are most common sources of unreliable specimens

Process of TB Samples Entering Lab

1. Digestion/ Decontamination
2. Smear- Fluorochrome
3. Inoculation of Media- Culture
4. MTD- if needed
5. Smear of culture growth- Carbofuchsin
6. TB complex probe
7. DST and Fingerprint if positive for TB complex
8. Identification of Non-tuberculosis mycobacteria

Smear vs. Culture

- Smear
 - Allows for early diag. b/c most mycobacteria grow at a slow rate
 - Used to monitor patients on treatment for conversion
 - Cannot be used in place of culture
 - Requires 5,000- 10,000 bacilli per ml of specimen to be recognized as positive
- Culture
 - Detects as few as 10- 100 viable mycobacteria

Digestion/Decontamination

- Digestion
 - Method used to release mycobacteria from clinical specimens (bodily fluids and cells)
 - Rapidly digests sputa
- Decontamination
 - Reduce/remove competing organisms (bacteria, yeast, etc.)

Initial Diagnosis of AFB

Fluorochrome Preliminary Smear

- Indicates the presence of some type of Mycobacteria
- Could be TB or NTM
(Non-tuberculosis Mycobacteria)

Graded from +/- to 4+

- +/- 1-2/ 300 fields
- 1+ 1-9/ 100 fields
- 2+ 1-9/ 10 fields
- 3+ 1-9/ field
- 4+ >9/ field



Media Used for Culture

All specimens inoculated into:

- LJ (Lowenstein Jensen) solid media
 - Scanned twice weekly for growth
- MGIT (Mycobacteria Growth Indicator Tube) liquid media
 - Fully automated incubation/monitoring system
 - Scanned once every hour for fluorescence indicating presence of growth

Mycobacteria Tuberculosis Direct Test (MTD)

- Performed on respiratory specimens only
- Detects amplified rRNA target from sediment of clinical processed specimens
- Target is *M. tuberculosis complex* sequences
- Similar to PCR
- Must be performed within 72 hours after processing
 - Specimen denatures allowing for false negatives

Requirements for MTD

Not performed on all specimens, must have at least on of the following:

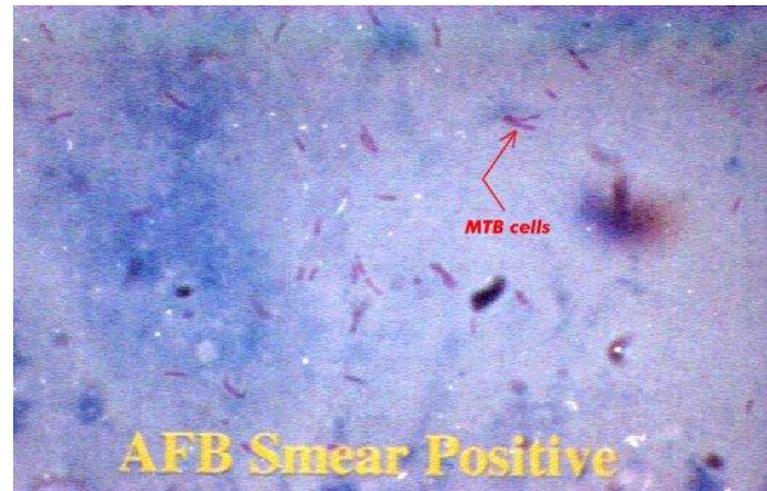
- Positive smear
- Positive skin test
- Abnormal chest x-ray
- Contact to TB patient
- * If patient has been on more than 7 days of TB meds MTD cannot be performed, result in false negative

Positive Culture Growth

Colonies of *M. tuberculosis* are dry, rough and buff in color on solid media

Carbolfuchsin Stain

- Hot Stain
- Indicates presence of AFB
- (Acid Fast Bacilli)
- TB or NTM



Gen-Probe Accuprobe

- “Culture” Confirmation Test
- DNA is labeled with a tag that fluoresces when measured with reader
- Identification of four species of mycobacteria
 - *M. tuberculosis complex*, *M. avium complex*,
M. gordonae and *M. kansasii*
- Allows for rapid identification of mycobacteria
 - Approx. 1 hour to complete test

TB Complex Probe

- *M. tb complex* probe consists of *M. tb*, *M. bovis*, *M. bovis BCG*, *M. canetti*, *M. africanum*, and *M. microti*
- In WV it is rare to find any organism other than *M. tuberculosis*
- Confirmation is done by positive Niacin biochemical test which eliminates the “complex” leaving only *M. tuberculosis*

Drug Susceptibility Testing

M. tuberculosis isolates that require DST are sent to PA Dept of Health, BOL

- First line drugs are performed initially
- Any resistance detected in first line are then set up for 2nd line drugs testing
- In WV there is low incidence of MDR or XDR TB
- Results are received from PA within 2 weeks for 1st line DST, less than 28 days for 2nd line

Genetic Fingerprint

All *M. tuberculosis* isolates are sent to Michigan Dept. of Community Health by WV OLS

- Gives a unique pattern for a particular organism
- Provides analysis which is helpful to TB Control to conduct epidemiology studies, link cases



Non-tuberculosis Mycobacteria (NTM)

Photochromogenic



Chromogenic



Identification of NTM

- Gen-Probe Accuprobe
 - *M. avium complex*, *M. goodsonae* and *M. kansasii*
 - Quick identification
- Biochemicals
 - May take 3 days to 3 weeks to complete test
 - * Piecing a puzzle together or finding the best possible match
 - * Referral to CDC if unable to identify at OLS

OLS- Strong Partner in Public Health

- CDC- provides grant funds & education
- APHL- Association of Public Health Laboratories provides funding for education
- DTBE- Division of TB Elimination, TB Control
- Local Health Departments- provides patient treatment and disease surveillance
 - Direct Observed Therapy (DOT)
 - TB clinics



GeneXpert® System



Real-time PCR based testing, performed all in one cartridge



GeneXpert® System

M. Tuberculosis complex detection and
Rifampin resistance detection
with one sample cartridge
- Eliminates cross contamination

Results in less than 2 hours





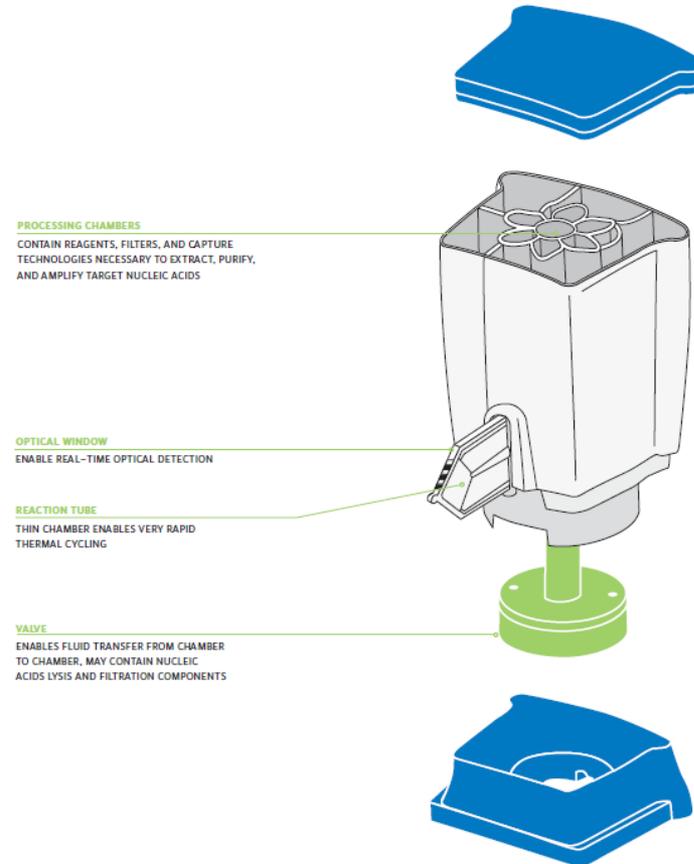
GeneXpert® System



-Fully automated system

-No human intervention once loaded in machine

Equipment is expected in house by March 2011
Approx. 4-6 weeks to validate, not FDA approved yet



Contact TB Unit

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TB Unit Lead Worker

nicole.d.haddox@wv.gov

304-558-3530 x. 2621

WV Office of Lab Services Website

<http://www.wvdhhr.org/labservices>